

CLAIMS

1. A Factor VII (FVII) or Factor VIIa (FVIIa) polypeptide variant having an amino acid sequence comprising 1-15 amino acid modifications relative to human Factor VII (hFVII) or human Factor VIIa (hFVIIa) having the amino acid sequence shown in SEQ ID NO:1, wherein said variant sequence comprises a substitution in at least one position selected from the group consisting of L39, I42, S43, K62, L65, F71, E82 and F275,

with the proviso that said variant is not

[K32E+D33N+A34T+K38T+L39E]hFVII/hFVIIa or

[A1Y+K32E+D33N+A34T+K38T+L39E]hFVII/hFVIIa or

[A1Y+A3S+F4GK+K32E+D33N+A34T+K38T+L39E]hFVII/hFVIIa or

[A1Y+L8F+R9V+P10Q+K32E+D33N+A34T+K38T+L39E]hFVII/hFVIIa or

[A1Y+A3S+F4GK+L8F+R9V+P10Q+K32E+D33N+A34T+K38T+L39E]hFVII/hFVIIa or

[A3S+F4GK+K32E+D33N+A34T+K38T+L39E]hFVII/hFVIIa or

[A3S+F4GK+L8F+R9V+P10Q+K32E+D33N+A34T+K38T+L39E]hFVII/hFVIIa or

[L8F+R9V+P10Q+K32E+D33N+A34T+K38T+L39E]hFVII/hFVIIa or

[I42N]hFVII/hFVIIa or [I42S]hFVII/hFVIIa or [I42A]hFVII/hFVIIa or

[I42Q]hFVII/hFVIIa.

2. The variant according to claim 1, wherein said variant sequence comprises at least one substitution selected from the group consisting of L39E, L39Q, L39H, I42R, S43H, S43Q, K62E, K62R, L65Q, L65S, F71D, F71Y, F71E, F71Q, F71N, E82Q, E82N, E82K and F275H,

with the proviso that said variant is not

[K32E+D33N+A34T+K38T+L39E]hFVII/hFVIIa or

[A1Y+K32E+D33N+A34T+K38T+L39E]hFVII/hFVIIa or

[A1Y+A3S+F4GK+K32E+D33N+A34T+K38T+L39E]hFVII/hFVIIa or

[A1Y+L8F+R9V+P10Q+K32E+D33N+A34T+K38T+L39E]hFVII/hFVIIa or

[A1Y+A3S+F4GK+L8F+R9V+P10Q+K32E+D33N+A34T+K38T+L39E]hFVII/hFVIIa or

[A3S+F4GK+K32E+D33N+A34T+K38T+L39E]hFVII/hFVIIa or

[A3S+F4GK+L8F+R9V+P10Q+K32E+D33N+A34T+K38T+L39E]hFVII/hFVIIa or

[L8F+R9V+P10Q+K32E+D33N+A34T+K38T+L39E]hFVII/hFVIIa.

3. The variant according to claim 2, wherein said substitution is L65Q.

4. The variant according to claim 2, wherein said substitution is F71Y.

5. The variant according to claim 2, wherein said substitution is K62E.

5 6. The variant according to claim 2, wherein said substitution is S43Q.

7. The variant according to any of claims 1-6, wherein said variant comprises at least two substitutions selected from the group consisting of L65Q, F71Y, K62E and S43Q.

10 8. The variant according to claim 7, wherein said variant comprises two substitutions selected from the group consisting of L65Q, F71Y, K62E and S43Q.

9. The variant according to claim 7 or 8, wherein said substitutions are selected from the group consisting of L65Q+F71Y, L65Q+K62E, L65Q+S43Q, F71Y+K62E, F71Y+S43Q and
15 K62E+S43Q.

10. The variant according to claim 1, wherein said variant comprises at least three substitutions selected from the group consisting L65Q, F71Y, K62E and S43Q.

20 11. The variant according to claim 10, wherein said variant comprises three substitutions selected from the group consisting of L65Q, F71Y, K62E and S43Q.

12. The variant according to claim 10 or 11, wherein said substitutions are selected from the group consisting of L65Q+F71Y+K62E, L65Q+F71Y+S43Q, L65Q+K62E+S43Q and
25 F71Y+K62E+S43Q.

13. The variant according to any of claims 1-12, wherein said variant comprises at least one amino acid modification in the Gla domain.

30 14. The variant according to claim 13, wherein said at least one modification in the Gla domain comprises a substitution in at least one position selected from the group consisting of P10, K32, D33 and A34.

15. The variant according to claim 14, wherein said variant further comprises an insertion of at least one amino acid residue between position A3 and F4.

16. The variant according to claim 14 or 15, wherein said substitution is made in position K32.

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17. The variant according to claim 16, wherein said substitution is K32E.

18. The variant according to any of claims 14-17, wherein said substitution is made in position P10.

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19. The variant according to claim 18, wherein said substitution is P10Q.

20. The variant according to any of claims 14-19, wherein said substitutions are made in positions P10 and K32.

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21. The variant according to claim 20, wherein said substitutions are P10Q+K32E.

22. The variant according to claim 15, wherein a hydrophobic amino acid residue is inserted between position 3 and 4.

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23. The variant according to claim 22, wherein said insertion is A3AY.

24. The variant according to any of claims 13-23, wherein no modifications are made in positions 6, 7, 14, 16, 19, 20, 25, 26, 29 and 35.

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25. The variant according to any of the preceding claims, wherein at least one amino acid residue comprising an attachment group for a non-polypeptide moiety has been introduced in a position located outside the Gla domain.

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26. The variant according to claim 25, wherein at least one non-polypeptide moiety is covalently attached to at least one of said attachment groups.

27. The variant according to claim 26, wherein said non-polypeptide moiety is a sugar moiety.

28. The variant according to any of claims 25-27, wherein said attachment group is a glycosylation site.

29. The variant according to claim 28, wherein said glycosylation site is introduced by
5 substitution.

30. The variant according to claim 29, wherein said introduced glycosylation site is an *in vivo* glycosylation site.

10 31. The variant according to claim 30, wherein said introduced *in vivo* glycosylation site is an O-glycosylation site.

32. The variant according to claim 30, wherein said introduced *in vivo* glycosylation site is an N-glycosylation site.

15 33. The variant according to claim 32, wherein said N-glycosylation site is introduced into a position comprising an amino acid residue having at least 25% of its side chain exposed to the surface.

20 34. The variant according to claim 33, wherein said N-glycosylation site is introduced into a position comprising an amino acid residue having at least 50% of its side chain exposed to the surface.

35. The variant according to any of claims 32-34, wherein said N-glycosylation site is
25 introduced by a substitution selected from the group consisting of A51N, G58N, G48N+S60T, T106N, K109N, G124N, K143N+N145T, A175T, I205S, I205T, V253N, T267N, T267N+S269T, S314N+K316S, S314N+K316T, R315N+V317S, R315N+V317T, K316N+G318S, K316N+G318T, G318N, D334N and combinations thereof.

30 36. The variant according to claim 35, wherein said N-glycosylation site is introduced by a substitution selected from the group consisting of A51N, G58N, G58N+S60T, T106N, K109N, G124N, K143N+N145T, A175T, I205T, V253N, T267N+S269T, S314N+K316T, R315N+V317T, K316N+G318T, G318N, D334N and combinations thereof.

37. The variant according to claim 36, wherein said N-glycosylation site is introduced by a substitution selected from the group consisting of T106N, A175T, I205T, V253N, T267N+S269T and combinations thereof.

5 38. The variant according to any of claims 32-38, wherein one N-glycosylation site has been introduced by substitution.

39. The variant according to any of claims 32-37, wherein two or more N-glycosylation sites have been introduced by substitution.

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40. The variant according to claim 39, wherein two N-glycosylation sites have been introduced by substitution.

41. The variant according to claim 39 or 40, wherein said N-glycosylation sites have been
15 introduced by substitutions selected from the group consisting of T106N+A175T, T106N+I205T, T106N+V253N, T106N+T267N+S269T, A175T+I205T, A175T+V253N, A175T+T267N+S269T, I205T+V253N, I205T+T267N+S269T and V253N+T267N+S269T.

42. The variant according to claim 41, wherein said N-glycosylation sites have been introduced
20 by substitutions selected from the group consisting of T106N+I205T, T106N+V253N, T106N+T267N+S269T, I205T+V253N, I205T+T267N+S269T and V253N+T267N+S269T.

43. The variant according to any of claims 32-37, wherein three or more N-glycosylation sites have been introduced by substitution.

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44. The variant according to claim 43, wherein three N-glycosylation sites have been introduced by substitution.

45. The variant according to claim 43 or 44, wherein said N-glycosylation sites have been
30 introduced by substitutions selected from the group consisting of T106N+A175T+I205T, T106N+A175T+V253N, T106N+A175T+T267N+S269T, T106N+I205T+V253N, T106N+I205T+T267N+S269T, T106N+V253N+T267N+S269T, A175T+I205T+V253N, A175T+I205T+T267N+S269T, A175T+V253N+T267N+S269T and I205T+V253N+T267N+S269T.

46. The variant according to claim 45, wherein said N-glycosylation sites have been introduced by substitutions selected from the group consisting of T106N+I205T+V253N, T106N+I205T+T267N+S269T, T106N+V253N+T267N+S269T and

5 I205T+V253N+T267N+S269T.

47. The variant according to any of the preceding claims, wherein said variant further comprises at least one modification in a position selected from the group consisting 157, 158, 296, 298, 305, 334, 336, 337 and 374.

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48. The variant according to claim 47, wherein said modification is a substitution selected from the group consisting of V158D, E296D, M298Q, L305V, K337A and combinations thereof.

49. The variant according to any of the preceding claims, wherein said variant further comprises at least one modification selected from the group consisting of K341Q, D196K, D196N, G237L and G237GAA.

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50. The variant according to any of the preceding claims, wherein said variant is in its activated form.

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51. A nucleotide sequence encoding a variant as defined in any of claims 1-50.

52. An expression vector comprising a nucleotide sequence as defined in claim 51.

25 53. A host cell comprising a nucleotide sequence as defined in claim 51 or an expression vector as defined in claim 52.

54. The host cell according to claim 53, wherein said host cell is a gammacarboxylating cell capable of *in vivo* glycosylation.

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55. A pharmaceutical composition comprising a variant as defined in any of claims 1-50, and a pharmaceutical acceptable carrier or excipient.

56. A variant as defined in any of claims 1-50, or a pharmaceutical composition as defined in claim 55, for use as a medicament.

57. Use of a variant as defined in any of claims 1-50 for the manufacture of a medicament for
5 the treatment of a disease or a disorder wherein clot formation is desirable.

58. Use according to claim 57, wherein said disease or disorder is selected from the group consisting of hemorrhage; uncontrolled bleedings, such as trauma; cirrhosis; thrombocytopenia; haemophilia A and haemophilia B.

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59. Use according to claim 58, wherein said disease or disorder is trauma.

60. Use according to claim 59, wherein said disease or disorder is blunt trauma.

15 61. Use according to claim 59, wherein said disease or disorder is penetrative trauma.

62. A method for treating a mammal having a disease or a disorder wherein clot formation is desirable, comprising administering to a mammal in need thereof an effective amount of the variant as defined in any of claims 1-50 or the pharmaceutical composition as defined in claim

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63. The method according to claim 62, wherein said disease or disorder is selected from the group consisting of hemorrhage; uncontrolled bleedings, such as trauma; cirrhosis; thrombocytopenia; haemophilia A and haemophilia B.

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64. The method according to claim 63, wherein said disease or disorder is trauma.

65. The method according to claim 64, wherein said disease or disorder is blunt trauma.

30 66. The method according to claim 64, wherein said disease or disorder is penetrative trauma.